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EQUIPMENT ACQUISITION

A Dupont Sorvall RC-5B centrifuge and medium and large volume rotors used for isolation of mitochondria and cytochrome oxidase has been installed and in use. A Mettler analytical balance has been obtained. An IBM PC computer with series and parallel ports, dual floppy disks and hard-disk drive have been obtained. A high resolution color monitor is yet to be purchased. An 8-channel A/D converter with 2 channel D/A output, 32Kb buffer and 40KHz acquisition time is being constructed to record and curve fit the kinetic data associated with CO and O2 binding to cyt oxidase and myoglobin. D/A output will allow use of the X-Y recorder already on hand without having to buy a digital plotter.

A low temperature chamber for measuring ligand binding to the heme proteins originally designed used dry N2 gas cooled via a copper coil immersed in liquid N2; this arrangement unfortunately consumed enormous quantities of liquid N2 and reached a temperature of only -70 degrees C. The chamber has been redesigned with a clear dewar and utilizes boil-off from liquid nitrogen; the system is economical and has been used down to -150 degrees. Xenon flash tubes have been purchased and are capable of photolyzing liganded Mb and cyt oxidase; the units are converted AC-powered camera flash units! The low temperature kinetic system at this point utilizes a 4-channel storage oscilloscope until the A/D unit is completed.

PERSONNEL

I have advertised for a post-doc without success thus far, but have received several recent inquiries; I have thus re-advertised (March 1 issue of SCIENCE) with hopes of securing the needed person. Since September 1, I have employed a full-time technician to assist in this research. Considerable progress has been made thus far.

TECHNICAL DATA

CYTOCHROME OXIDASE

Since Jan 1,1985 we have been measuring the Km and Vmax parameters of cytochrome oxidase in intact mitochondria in the presence and absence of lidocaine, tetracaine, and procaine anesthetics. We have previously determined that 50% inhibition of succinate oxidase activity occurs with 5 mM tetracaine and 50 mM lidocaine; we observed no inhibition of cytochrome oxidase at 10 mM lidocaine (expected 30%) and only 10% (expected 90%) with 10 mM tetracaine. This suggests that cytochrome oxidase is not as sensitive to these compounds as is succinate oxidation. However, we have determined that 10 mM tetracaine but not procaine or lidocaine increases the Km 5-fold without an increase in Vmax; this suggests that tetracaine is a competitive inhibitor of cytochrome oxidase and explains the observed inhibition by other workers. At a fixed cytochrome c concentration, as the Km increases, the velocity of the reaction must necessarily decrease! Other workers have reported only the apparent inhibition, not the increase and Km, which adequately explains their findings. The molecular basis for the increase in Km of cyt c is not apparent except that it is not a charge or ionic strength effect.

Early findings in intact mitochondria indicate that tetracaine and procaine, but not dibucaine or lidocaine, cause shifts in the 604 nm alpha band of cytochrome oxidase. Also, dibucaine, tetracaine, and lodocaine cause 20-30 mV increases in apparent midpotential of the oxidase in mitochondria. We have not yet studied isolated oxidase.

MYOGLOBIN

NMR

We have spent considerable time on myoglobin. While not as well characterized as cytochrome c in terms of H-NMR, we have some interesting early data thus far. We have done two types of experiments. The first investigates changes in the protein by anesthetics. Thus far, procaine, tetracaine, and dibucaine have not caused noticeable changes at 5 mM concentration. Lidocaine, however, causes a significant change in the 3.7 ppm resonance (unidentified at this writing).

The second type of experiment assays changes in the anesthetic due to interaction with myoglobin. No changes were observed with procaine, but dramatic changes were observed with lidocaine with less prominent changes in dibucaine and tetracaine. I am in the process of identifying the part of the molecule being altered and thus interacting with Mb. This data is somewhat similar to that with cyt c, where procaine was without effect but lidocaine caused noticeable changes in protein resonances.

EPR

Preliminary results have begun on changes in EPR resoances by these molecules. Mb has resonances at g=6 and g=2, with a small resonance at g=2.2. Lidocaine and dibucaine change the g=2 but not g=2.2 signal, while tetra-, procaine, and procainamide abolish the g=2.2 peak. Lido- and tetracaine alter the g=6 signal. In general, all anesthetics increase the intensity of the resonances. These results are encouraging and provide evidence of altered Mb structure.

VISIBLE SPECTRA

Alteration of the 411 nm (Mb) and 423 nm (MbO2) peaks has also been studied. In general, all drugs tested (lido-, dibuc-, tetra-, procaine and procainamide) induce changes in the 411 peak to a shorter wavelength. Procaine and tetracaine show approximate 1 nm shifts in the MbO2 423 peak. We have not yet analyzed the data to determine changes in peak height or bandwidth, indications of changes in extinction coefficient.

CYTOCHROME c

EPR

Cytochrome c has resonances at g=3 and g=2.2 that we are studying. No compound alters the position of g=2.2, but all narrowed the half-bandwidth (narrowed the signal) without an increase in peak height; in other words, a change in "microwave extinction coefficient" is not observed. Significant changes in g=3 position were observed with all 4 -caine anesthetics, but only dibucaine decreased the bandwidth. Only native cyt c is affected; denatured cyt c (with g-values of 2 and 4.3) are unaffected, and are used as an internal standard for peak position.

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Hand-calculating the copious data is time-consuming; we await the imminent arrival of the interface unit to allow the IBM 9000 computer to handle the EPR data (machines provided by Physics Dept).

VISIBLE SPECTRA

Data presented in the original proposal indicated a change in cyt c extinction coefficient. We have further evidence that indicates that lidocaine, procaine, propranolol, and procainamide (cardiac antiarrhythmics) decrease the extinction coefficient, while dibucaine, tetracaine, and verapamil do not cause statistically significant effects.

While the equipment has been in place for less than 4 months, I feel that we have made excellent progress in the preliminary aspects of this study. I do not anticipate changes in the proposed research techniques or procedures. Preliminary evidence indicates that we are headed in the proper direction and that results are being obtained, although the significance and relationships to each other warrant further investigation. I am hopeful that the post-doctroral position will be filled by summer and that we can proceed at a faster rate; in the event the position is not filled, I plan to continue with graduate asistant/technician performance of the experiments. I am pleased with our progress to date.